were challenged by in vivo collagenase treatment, increased staining of the chondrocytes was seen.
Conclusions: We identified a genetic variant on chromosome 19 to be associated with cartilage thickness and hip OA. The variant is located in a gene showing an expression pattern that supports a role in chondrogenetic differentiation. GWAS for underlying endophenotypes might be a prolific route to identify risk loci for complex diseases such as OA.

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GLOBAL DNA METHYLATION ANALYSIS IN OSTEOARTHRITIS SYNOVIAL FIBROBLASTS

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Purpose: Aberrant transcription profiling in osteoarthritis(OA) synovial fibroblasts(SFs) lacking consistent specific genetic mutations suggests that epigenetic mechanisms along with the environmental factors may be involved in its pathogenesis. Compared to those from normal or rheumatoid arthritis(RA) SFs, over-activated OASFs have an important role in the destruction of normal joint architecture, and the imprinted, phenotypical aggressiveness could be determined by epigenetic mechanism. To investigate a global DNA methylation of human OASFs using methylated DNA isolation assay (MeDIA)-CpG promoter microarray, and compared it with SFs of RA patients or healthy controls in Korean population.
Methods: Human synovial tissue samples obtained during undergoing total knee joint replacement surgery in 2 OA and 3 RA patients. 2 Normal synovium was collected by arthroscopic or open knee surgery of traumatic ligament injury of healthy populations. Total DNA extracted from SFs using the QIAamp DNA mini and blood kit protocol (Qiagen, Hilden, Germany). To discover novel hypo- or hypermethylated genes in OA by genome-wide search, we introduce a MeDIA-coupled CpG microarray method for directly identifying differentially methylated regions of the genomes in each pooled synovial cells between OA and RA patients. The methylation status of promising candidates was validated by quantitative pyrosequencing assay in each synovial cells.
Results: In two CpG microarray, 4 genes were screened as 5 fold hypomethylated targets among 1,714 porbes. Through stepwise subtraction processes, we finally selected four candidate targets. Among of these targets, two genes, *APEX1 gene(gt-OA1)* and *TGFB1 gene(gt-OA2)* have shown so far a significant decrease in the methylation frequency in OA when compared in independent groups of synovial DNA samples from OA patients with from RA and normal controls.
Conclusions: *APEX1 and TGFB1 gene* promoter is hypomethylated in OASFs than OA and healthy controls. Pathophysiologic correlation and their role as a diagnostic or prognostic marker should be investigated in a larger number of patients group hereafter.

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HFE C282Y HOMOZYGOSITY IS ASSOCIATED WITH AN INCREASED RISK OF TOTAL HIP REPLACEMENT FOR OSTEOARTHRITIS IN MEN BUT NOT WOMEN

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Purpose: The evidence for an association between mutations in HFE gene related to hemochromatosis and risk of hip or knee osteoarthritis is inconsistent. Total joint replacement is considered a surrogate measure for symptomatic end-stage osteoarthritis. This study aimed to examine the relationship between HFE gene mutations and risk of total hip and knee replacement in a population-based cohort.
Methods: The Melbourne Collaborative Cohort Study is a prospective cohort study that commenced recruitment in 1990. Participants born in Australia, New Zealand, the United Kingdom, or Ireland (n = 27,848) were genotyped for the HFE C282Y variant. Total hip and knee replacements for osteoarthritis during 2001–2009 were ascertained from the Australian Orthopaedic Association National Joint Replacement Registry. Hazard ratios (HRs) and confidence intervals (CIs) were obtained from Cox regression.
Results: Compared to those with no C282Y variant, C282Y homozygotes were at increased risk of total hip replacement (HR 1.94, 95% CI 1.04–3.62). The association was stronger for men (HR 3.34, 95% CI 1.48–7.52) than for women (HR 1.22, 95% CI 0.46–3.27) (p for interaction = 0.21). Only 3 C282Y homozygotes had total knee replacements; the HR was 0.51 (95% CI 0.16–1.57). C282Y/H63D compound heterozygosity was not related to the risk of total hip or knee replacement.
Conclusions: HFE C282Y homozygosity was associated with increased risk of total hip replacement for osteoarthritis for men but not for women. The mechanism for this is unknown and the findings need to be confirmed in future studies.

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THE mtDNA HAPLOGROUPS INFLUENCE THE PROGRESSION OF OSTEOARTHRITIS (OA)

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Purpose: To analyze the influence of the mtDNA haplogroups in the progression of the osteoarthritis (OA) disease.
Methods: The DNA of 282 knee and/or hip OA samples from Hospital Universitario A Coruña was isolated to obtain their mtDNA haplogroups. Knee and/or hip radiographs from all these samples were obtained at two stages of the OA disease of at least 36 months between them, and evaluated according to the K/L scale from grade 0 to IV. Statistical analyses included Kaplan-Meier survival curves for each haplogroup or cluster tested, as well as Cox regression models taking into account another variables such as gender, haplogroup (or cluster) and body mass index (BMI).
Results: For this study, two types of progression were established: OA progression and severity progression. We considered OA progression when a patient evolved from K/L grade 0-I to K/L grade II-III or K/L grade IV, and from K/L grade II-III to K/L grade IV, in at least one of the joints analyzed. The results obtained showed that the OA progression varies significantly according to the mtDNA haplogroups (p = 0.030), and the main differences were detected when compared patients that belonged to the cluster TJ (better progressors) with patients of the cluster KU (worse progressors) (p = 0.083) (Figure 1). On the other hand, we considered severity progression when a patient evolved to knee or hip prosthesis from an initial K/L grade III or less. In this case, the results obtained showed that patients carrying the most common mtDNA haplogroup H had a worse severity progression than non-H patients (p = 0.035) (Figure 2). The Cox regression model also showed that males evolved worse than females (p = 0.028).
Conclusions: The mtDNA haplogroups influence the progression of OA; these results strength the role of the mitochondria in the OA disease.

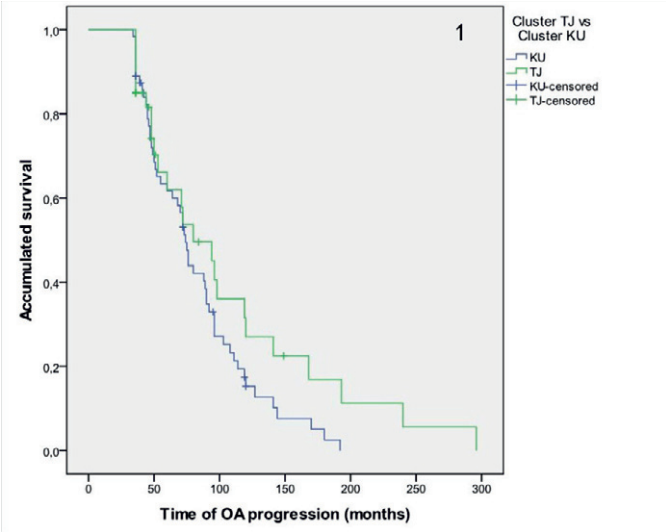

Fig. 1. Kaplan–Meier survival curve showing the different OA progression between clusters KU and TJ.

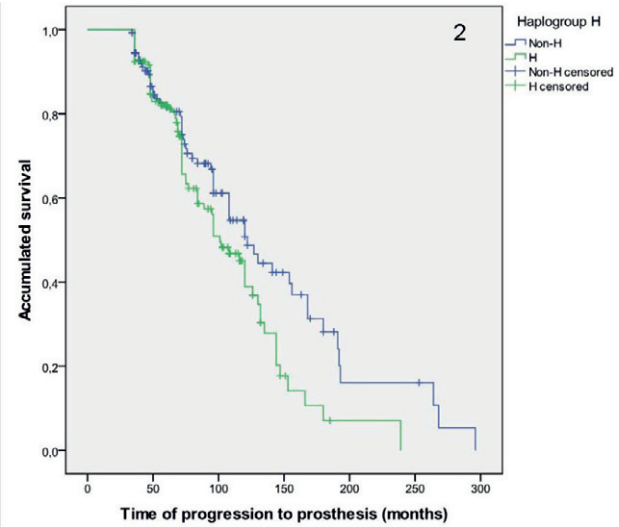

Fig. 2. Kaplan–Meier survival curve showing the different severity progression between carriers of the haplogroup H and non-H carriers.

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THE ASSOCIATION OF GENES WITHIN THE VITAMIN D METABOLISM PATHWAY AND RADIOGRAPHIC KNEE OSTEOARTHRITIS: DATA FROM THE OSTEOARTHRITIS INITIATIVE GENOME-WIDE ASSOCIATION STUDY

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Purpose: Previous epidemiologic studies have demonstrated an association of low serum 25-hydroxyvitamin D levels with the development and progression of osteoarthritis (OA) of the hip and knee. Potential pathophysiologic mechanisms include effects on chondrocyte metabolism and immune responses as well as associations with low bone mineral density. A prior genome-wide association study (GWAS) demonstrated associations of single nucleotide polymorphisms (SNPs) in genes involved in vitamin D metabolism with serum 25(OH)D insufficiency (Lancet 2010;376:180–8). The present analysis focused on whether SNPs in genes within the vitamin D metabolism pathway are associated with radiographic knee OA.

Methods: We tested for associations between radiographic knee OA (RKOA) and 271 SNPs falling within 6 Vitamin D metabolism pathway genes (*CYP2R1*; *CYP24A1*, *CYP27A1*, *CYP27B1*, *GC*, *VDR*) in 3286 participants from the Osteoarthritis Initiative (OAI). Cases (N = 2209) had at least one knee with definite osteophytes irrespective of the presence of joint space narrowing (equivalent to Kellgren-Lawrence [KL] grade 2 of higher). Controls (N = 1077) had both knees free of both osteophytes and joint space narrowing (equivalent to KL grade 0). The candidate gene SNPs were genotyped on the Illumina 2.5M platform as part of the GWAS of OAI participants. Odds ratios for the association of individual SNPs and RKOA were estimated from logistic regression analyses adjusted for age and gender; analyses were conducted separately for Caucasians and African-Americans. We estimated that the sample size would provide 80% power to detect associations with odds ratios of 1.25–1.37 for SNPs with minor allele frequencies of 0.10 to 0.50 ($\alpha = 0.001$).

Results: Demographic features of cases and controls are shown in Table 1. In single SNP analysis of 2,669 Caucasian subjects, RKOA was nominally associated with 2 SNPs in *CYP2R1* ($p < 0.05$), 1 SNP in *VDR* ($p < 0.02$), and 1 SNP in *CYP24A1* ($p < 0.02$). Using a gene-wise permutation test that accounted for correlations among SNPs and the number of SNPs tested within each gene, none of these associations was smaller than what would have been expected by chance. In single SNP analysis of 617 African-American subjects, RKOA was nominally associated with 2 SNPs in *CYP27A1* ($p < 0.03$), 1 SNP in *VDR* ($p < 0.03$), and 2 SNPs in *CYP24A1* ($p < 0.03$). After performing the gene-wise permutation test, there remained weak evidence that the association observed between *CYP24A1* SNP20–52212617 and RKOA ($p = 0.007$) was smaller than would have been observed by chance after taking into account the multiple testing within this gene ($p = 0.06$).

	European Caucasians		African Americans	
	Cases (n = 1807)	Controls (n = 862)	Cases (n = 402)	Controls (n = 215)
% male	44.9	41.1	28.4	36.3
Age (yrs)	62.9 ± 9.2	59.4± 9.0	60.1 ± 8.4	57.0 ± 8.0
BMI (kg/m^2)	28.9 ± 4.6	27.1± 4.4	32.2 ± 4.9	29.3 ± 4.5

Conclusion: These are the first preliminary results from the Genome wide association study of participants in the OAI. They suggest that, despite weak evidence of an association of a single SNP within the CYP24A1 gene in African-Americans, genes within the vitamin D metabolism pathway do not appear to influence the development of RKOA in U.S. adults.

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GENOME WIDE EXPRESSION ANALYSIS OF OSTEOARTHRITIS AFFECTED AND PRESERVED CARTILAGE FROM JOINT REPLACEMENT SURGERY MATERIAL IN THE RAAK STUDY

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Purpose: To chart the genes and processes involved in the breakdown of cartilage during osteoarthritis (OA) in a genome wide expression approach of a considerable number (N = 33) of matched OA affected and preserved cartilage samples using microarrays.

Methods: For 33 patients of the research osteoarthritis and articular cartilage (RAAK) study (mean age 66.2, range 54–80) we collected paired cartilage samples at joint replacement surgeries. Affected (near lesion) areas were matched by preserved (distant from lesion) areas within each single joint. We included both hip (N = 22) and knee (N = 11) replacements. By use of microscopic assessment a scoring of the cartilage damage was made according to Mankin which takes both cellular and matrix features of OA into account. RNA was isolated by use of cryogenic disintegration of the cartilage followed by Qiagen column extraction and checked for quality (RIN > 8.0) and quantity by lab-on-a-chip techniques before applying the standard protocol for Illumina microarray measurements on the HT12-v3-microarrays. After microarray analyses all 33 sample pairs passed basic quality control. Analysis of the data was performed using 'R' and the Limma package identifying the most significantly up- or downregulated single genes, corrected for multiple testing (Holm's method).

Results: Although for some samples the preserved areas did show signs of OA damage, the Mankin grading for cartilage indicated overall less damage within the preserved areas as compared to the matched affected cartilage areas. In total, we detected 206 probes (representing 183 genes) which were differentially expressed with a Holm adjusted P-value <0.05, irrespective of fold changes of expression. Amongst these were genes associated to inflammation, the complement system, development, matrix associated genes and several genes involved in neuronal processes.

When we prioritized the data for genes that showed a fold change of at least 2, we found the expression to be decreased for 5 genes and increased for 15 genes in affected as compared to matched preserved cartilage (23 corresponding probes). The markedly up-regulated genes belong to pathways which are the usual suspects for involvement in OA being; inflammation (prostaglandin E synthase (*PTGES*), chemokine ligand 14 (*CXCL14*)) and developmental or matrix associated genes (osteopontin (*SPP1*), frizzled related protein (*FRZB*), collagen type IX alpha 1 (*COL9A1*)). More remarkable, we observed extensive and significant up-regulation of the nerve growth factor (*NGF*) gene.

Conclusions: This is the first analysis of micro-array expression data of a large number of OA affected cartilage samples matched by preserved cartilage from the same joint. We show differential expression 183 genes reflecting several processes that may mark the ongoing OA process in human cartilage. Possibly, amongst these genes are potent therapeutic targets which may be targeted to stop the process of cartilage breakdown.